GENETICS

The culmination of a decade of fastidious experiments by Arthur Kornberg and many associates at Stanford University, Stanford, Calif., resulting in the *in vitro* replication (duplication in a test tube) of a deoxyribonucleic acid (DNA) virus was a cardinal event in the history of biology. It overshadowed the broad progress that research in genetics had been making in recent years. Reported in mid-December 1967 by Professors Kornberg, Mehran Goulian, and Robert Sinsheimer, the historic importance of the event was unchallengeable.

By 1959 Kornberg had been recognized with a Nobel Prize for his discovery of the enzyme DNA-polymerase. The properties of this enzyme made it virtually certain that it played a central role in the assembly of DNA and in the natural process of gene replication within the cell. Many technical obstacles, however, had to be cleared in order to prove conclusively that genetic information could indeed be accurately copied by a simplified enzyme system in which the functions of each component were clearly understood. (*See* Feature Article: THE LANGUAGE OF LIFE.)

One of the important aspects of Kornberg's experiments was the choice of the DNA of a particular type of virus, a bacteriophage called ϕX 174, or phi ex. Unlike most DNA, which is found in complementary double-stranded fibers, the DNA of liberated ϕX-phage particles is single-stranded and circular, the ends of the molecule being joined. (*See* Year in Review: MOLECULAR BIOLOGY.)

In Vitro Replication

For the biochemist, the physical distinctiveness of circular DNA provides a special opportunity to isolate the native viral DNA in remarkably purified form, free of any host DNA or any broken fragments of the virus. Kornberg and his associates were able to use highly purified DNA, carefully labeled with radioactive isotopes, as the input primer. Two cycles of DNA-polymerase action were needed to produce a double-stranded DNA with complementary circles and then to separate and use these circles as templates for the synthesis of recomplemented circles that would have the same composition, genetic information, and biological activity (infectivity) as the original DNA. As verified by isotopes the progeny virus that resulted had none of the labeled atoms of the primer.

The net result of these experiments, then, was the replication, under controlled conditions in the test tube, of a viral DNA strand that should be sufficient to determine the code for about six to ten genes. Six genes had been identified, and much had been learned about each of their functions in the virus by early 1968. In this respect, while ϕX was one of the simplest organisms, it had become the most thoroughly understood genetically. The demonstrated replication of the DNA meant that the enzymatic process must have been very nearly perfect for the entire length of the strand, or for roughly one one-millionth of the genetic information present in every cell of a higher animal.

Following the Kornberg success, A. T. Ganesan, also at Stanford, achieved a similar result in the replication of fragments of bacterial DNA. Although this was a much more difficult and less elegant system than the one first used by Kornberg, it demonstrated that the same principles could be used to achieve the artificial replication of a double-stranded DNA. The test of biological activity for double-stranded DNA, however, was to replace mutant genes with effective DNA that had been copied by a DNA-polymerase system isolated from the cell membranes of the corresponding bacteria.

Significance of the Studies

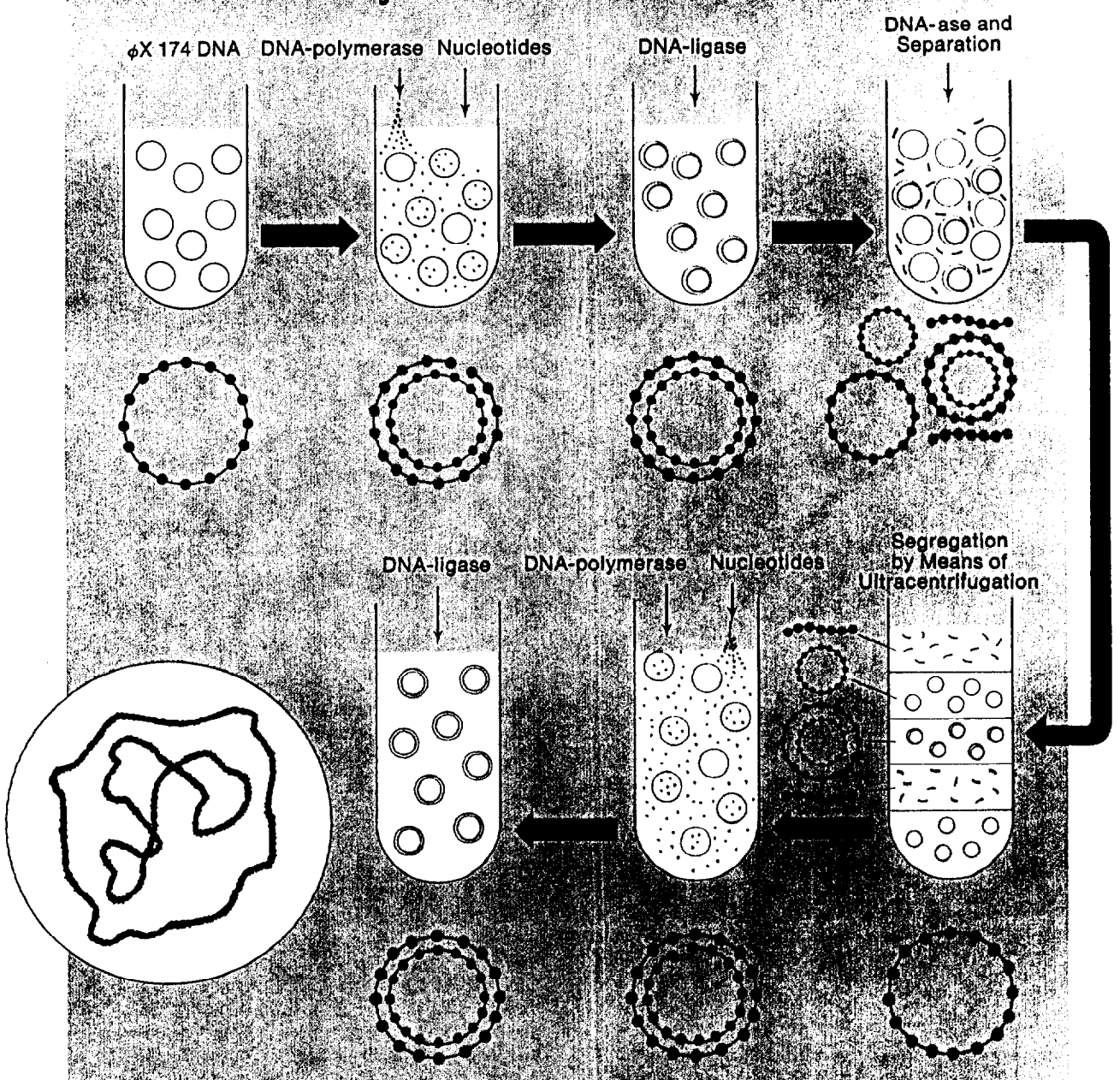
These achievements abolished any lingering doubt that the replication of genetic information could be understood in terms of relatively simple processes of enzyme chemistry. The fundamental step in DNA synthesis, the copying of genetic information, is the assembly of a large macromolecule from an assortment of four building blocks, the nucleotides of adenine (A); thymine (T); guanine (G); and cytosine (C). To insure accurate replication of the DNA, it is necessary that the assembly of these building blocks proceeds in an orderly linear fashion; each building block must follow strictly the sequence of the parent macromolecule.

To apply generalizations from these findings to the entire living world would require additional experimentation, especially because little is known of the molecular architecture of the chromosomes of higher organisms. The physicochemical structure of DNA from mammalian and human sources, however, does not differ fundamentally from that obtained from plants or from single-celled organisms and viruses—even though each particular DNA has a unique information content that distinguishes one organism from another.

"Replication," not "Creation"

News of these scientific advances aroused widespread interest, extending even to the drafting of legislation to establish government commissions to investigate the social, moral, ethical, and legal implications of such discoveries. It is,

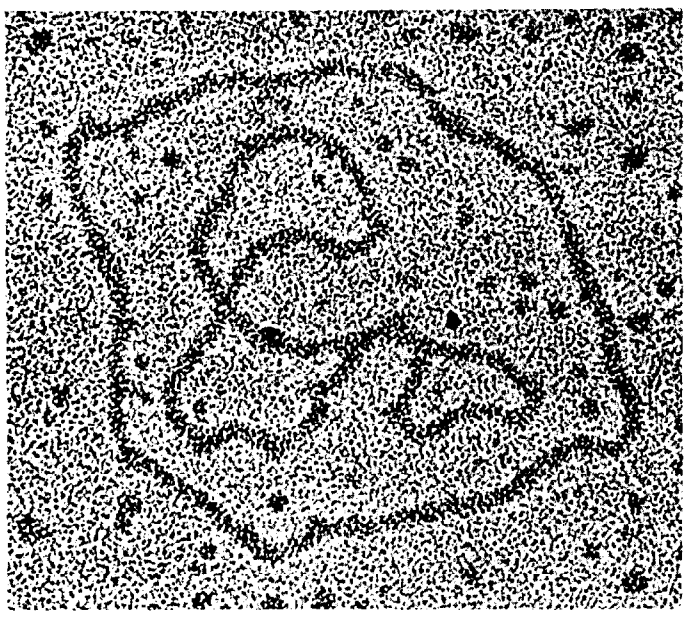
The *in vitro* Synthesis of Viral DNA



To produce a synthetic, biologically active replica of DNA, Stanford geneticists used the circular DNA of the φX 174 virus. The enzyme DNA-polymerase, which causes formation of a complementary polypeptide chain, and nucleotides containing the four building blocks of DNA were added to the test tube solution. Because the synthetic circles were not complete and, hence, not biologically active, it was necessary to add the newly discovered enzyme DNA-ligase to join the strands. The biological activity of the synthetic circles was established as follows: A third enzyme, deoxyribonuclease (DNA-ase), was used to open the circles, and the solution was heated so that the structures would separate. By means of centrifugation, the separated chains and linear forms of both natural and synthetic DNA were segregated so that the synthetic chains could be isolated and treated as the natural ones had been. A second, completely synthetic round of replication was then induced.

of course, a far cry from a scientific demonstration of basic principles—no matter how important they may be for philosophical insight—to the development and dissemination of techniques for practical exploitation.

Much of this interest was stimulated by news reports of these developments appearing under such headlines as "Creation of Life in a Test Tube." The words "creation" and "life," however, had no precise definitions in this context; as Kornberg meticulously pointed out, they played no part in the scientific analysis of the problem. A virus particle can, however, be regarded as an autonomous, living organism, even when it can only "grow" in a very complex medium simulating the interior of a bacterial host cell. The promptness with which a double-



An electron micrograph shows two single-stranded viral DNA rings synthesized in the test tube at Stanford University School of Medicine. The actual length across the DNA is two microns (two millionths of a meter).

stranded bacterial DNA fragment could also be replicated *in vitro* indicated that the synthesis of a whole bacterial nucleus would require only a thousandfold greater effort of the same kind; a mammalian cell nucleus would require a millionfold effort. This principle having been well established, it would be of questionable value to invest the enormous effort to repeat the whole task as a strictly chemical exercise.

The replicated ϕX virus was just that, a replica of an existing virus, and not a "creation." The experiments were directed toward understanding how replication takes place in the cell; the *in vitro* production of ϕX was the surest proof that the process was understood.

There was no doubt that this knowledge opened the door to new inventions such as viruslike particles with structures designed by human intelligence. Indeed, such "creations" (namely artificial sequences of nucleotides assembled as DNA) had been manufactured for several years by Kornberg and others. Artificial polymeric sequences, with designations such as ATATATAT and GGGGGG because of their monotonous, simple structure, had played a crucial part in theoretical studies of DNA *in vitro*. When the special conditions under which cells can be infected with such polymers are understood, it might then be possible to say that an artificial protovirus has been "created," even if a natural one had not been replicated *in vitro*.

Two barriers to new "creations" remained: (1) sufficient knowledge of the details of sequences that would be effective as a virus or as a gene, and (2) techniques to fabricate such sequences by design; *i.e.*, from a conceptual blueprint rather than from an existing natural prototype. Both barriers were being rapidly breached. Most new advances, however, would make preeminent use of natural genes and organisms in which selected parts would be modified by *in vitro* methods.

Popular Concerns

By itself, *in vitro* replication of DNA, whether viral or human, scarcely raises any issues of public policy. It accomplishes nothing that does not already take place on a vastly larger and more complex scale in all living cells, whether they are parts of intact organisms or isolated in artificial tissue cultures.

Statesmen were wise, however, to infer that *in vitro* replication symbolized a revolutionary advance in biological insight, the breaching of superstitious reservations about the interpretation of living processes in terms of chemistry and physics. There was little doubt that these and subsequent biological discoveries would open many new options, and with them the dilemmas of conscious choice, in areas that had been beyond man's influence.

Nevertheless, it was proper to ask whether "genetic engineering" was a prospect for the near future. I have been seriously questioned as to when we could expect to breed "supermen," and cautioned that they be produced in America before the Chinese learn the knack. If we knew enough about human nature to identify and design a valid superman, and still wanted to produce one, the finesse of *in vitro* DNA replication would be almost superfluous: no DNA could make an infant impervious to the quality of his education. Conversely, by the time this would be possible, the scientific basis for the intelligent application of genetic engineering

already would have revolutionized education with new insights into human development and its direction.

Some commentators were concerned lest biological engineering get into the hands of another Hitler. Totalitarian regimes scarcely need such sophisticated tools; the problem of defending individual freedom is hardly altered by details of this new biological knowledge.

Possible Applications

Developments in DNA biology would undoubtedly have dramatic applications, particularly in the study and control of viruses for the production of safe, effective vaccines and for the development of antiviral drugs. They would certainly play a key role in the attack on many diseases in which viruses are important, though sometimes obscure: cancer, birth defects and mental retardation, autoimmune diseases, and progressive deterioration of the brain. Indirectly, DNA virus studies would have a role in tissue and organ transplantation and in examining the biology of aging. Viruses are often exquisitely specific for certain tissues; I can visualize using them even for complex therapeutic purposes like clearing arteries of deposits of cholesterol, or for enhancing the circulation of the blood in the heart or the brain.

A primitive level of viral engineering was already developing specific disease-causing viruses that can control crop-damaging insects or be used as innocuous competitors of natural pathogens (disease-causing organisms). (*See* Year in Review: AGRICULTURE.) For higher organisms there was as yet no clue as to how to find a specific gene on an intact chromosome of a given cell and replace it with another. Finding the correct one ten-millionth of the total DNA seemed an implausible aim. Because a wide margin of error would be intolerable, direct viral engineering of the human merited little discussion.

Infants born with specific genetic defects may, however, be treated by the use of engineered viruses in the form of avirulent vaccine-like agents to which restorative genetic information had been grafted and carefully checked. Avirulent viruses are already widely used for vaccination to provoke immunity to smallpox, polio, and measles. They are the historic forerunners of DNA agents yet to come that may be used to improve the human condition.

Spontaneous mutation was still regarded as inevitable in its primary occurrence and in the eventual toll that it exacts in the form of genetic disease and fetal death. Exact knowledge of DNA replication, however, would soon dispel much of the mystery surrounding the mutation process and show how it could be intelligently controlled. A great deal was already known about how radiation damages DNA and intracellular repair mechanisms. DNA studies were also providing the basis for warnings about a number of chemical compounds that contaminate the environment and that may play an important role in the deterioration of human genes.

One of the most spectacular results of DNA biology may very well be its use in preventing rejection of transplanted organs. Many workers believed that the solution to the transplantation problem would be the large-scale production of the genetically determined substances that would line the surface of cells used in transplantation. These substances, when used in excessive amounts and in company with certain drugs, might provoke excessive paralysis of the rejection response when tissue coated with them was implanted in a genetically different host. The body's immune responses to other foreign material would be left intact. A specific blockade of the immune reaction would require a repertoire of these provocative antigens. The isolation of one antigen known to produce transplant rejection in humans was announced in May 1968.

—Joshua Lederberg

See also Year in Review: BIOPHYSICS, *Radiation Biology;* DRUGS; MOLECULAR BIOLOGY; ZOOLOGY.

GEOLOGY, GEOCHEMISTRY, AND GEOPHYSICS

There never has been a more exciting time for earth scientists than today. The whole field is in explosive growth. New techniques are revealing new areas of research and new observations are leading to new and controversial theories of the earth. At the same time, the study of the origin, history, and nature of the earth is becoming less observational, or purely descriptive, and more experimental and quantitative. (*See* Feature Article: STUDYING THE EARTH.)

This is particularly true of those aspects of geology that relate the structure of the earth's crust to processes going on in the deeper layers of the earth. There is an ever-increasing amount of evidence that the distribution of continents and ocean basins, mountain chains,